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16. ABSTRACT

Urease was treated in vitro with 93 chemicals of various chemical classes in order to study inhibitory effects. The organometallic chemicals, methyl mercuric chloride and phenyl mercuric chloride were the strongest inhibitors studied, about 10^{-7} times more than NaCl. Also highly inhibiting were Hg(II), Ag(I), and Cu(II), followed by IO_4^{-1}, Au(III) and Cd(II). In addition, the anions $Cr_2O_7^{-2}$, F^{-1}, AsO_2^{-1}, and BH_4^{-1} had a moderate effect. No measurable effect was shown by the anions, Br^{-1}, I^{-1}, N_3^{-1}, and S^{-2}. The herbicide, 2,4-D, was found to be the most reactive of the organic chemicals studied, followed closely by sodium dodecylsulfate, 2,2-dichloropropionic acid and p-chloro-benzoic acid. The herbicides derived from urea (diuron, monuron, and fenuron) were found to be unreactive. Of the following multi-valent chemicals tested, selenite was more inhibiting than selenate; Pd(II) more than Pd(IV); arsenite more than arsenate; Cr(VI) more than Cr(III) and Fe(II) more than Fe(III). None of the widely used pesticides such as malathion, captan, diazinon, and carbaryl elicited a measurable effect. Warfarin (rodenticide) and pentachlorophenol (fungicide) caused a mild effect. In two preparations of mixtures made up of ten components each, an approximately 10-fold increase in inhibition was found, indicating that for the chemicals tested, the toxic effect was additive.

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Effect of Selected Environmental Pollutants and Other Chemicals on the Activity of Urease (*in vitro*)

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A wide variety of chemicals have been found to affect the activity of the enzyme, urease, (urea amidohydrolase, E.C. 3.5.1.5). It has been found to be strongly inhibited by many transition metal cations (SUMNER & SOMERS 1953; SHAW 1954; SHAW & RAVAL 1961; TOREN & BURGER 1968; HUGHES et al. 1969). The literature also indicates that urease is inhibited by other classes of chemicals, including certain organochlorine insecticides (TSIRKOV 1969), substituted urea herbicides (CERVELLI et al. 1975, 1976, 1977), organophosphorus insecticides (LETHBRIDGE & BURNS 1976), and organomercurials, ethacrynic acid and penicillamine (KUNIN 1976). Drugs have also been screened for effect on urease in vitro (BURR 1977). Polyphenols cause some degree of inhibition (FERNANDO & ROBERTS 1976) as do certain triazoles (GAUTHIER et al. 1976) and several heterocyclic mercaptans (GOULD et al. 1978). Further, organic trivalent arsenicals inhibit the enzyme (GORDON & QUASTEL 1948) as do certain anionic detergents and related compounds (WILLS 1954) and hydroxamic acids (KOBASHI et al. 1975). Mercuric ion evidently is the most inhibiting of all chemicals tested.

Because of the sensitivity of urease to many foreign chemicals, it is a useful indicator for the presence of certain reactive chemicals in field situations, where, for example, it has been analyzed in soil biota to reflect the presence of fertilizers, herbicides, pesticides and environmental pollutants (TYLER 1974; MISHRA & FLAIG 1979). This enzyme in the immobilized state was also used for the determination of the presence in the environment of trace amounts of heavy metal ions such as mercury (OGREN & JOHANNSON 1978). It is proposed for use for the detection of any type of urease inhibitor in contaminated water (CHRISTENSEN & RIEDEL 1980).

The current work was undertaken to determine the relative inhibitory effect on urease by a large group of water pollutants, environmental contaminants and certain additional chemicals. In addition, two preparations were studied in order to simulate water containing mixtures of chemicals, one containing 10 inorganic and the other 10 organic components.

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MATERIALS AND METHODS

Urease activity was determined by monitoring the rate of increase in pH when lightly buffered urea was catalytically hydrolyzed producing ammonium carbonate, following an established procedure (HUGHES et al. 1978; KOBASHI et al. 1975; BURR 1977; GOULD et al. 1978). The reaction rate was monitored for 2.5 min as the pH changed from about 6.00 to about 8.50. Concentration values for amounts of NH_4^+ generated were derived from a standard curve prepared from plotting known NH_4OH concentrations in the reaction media vs. pH. Urease (10.0 mg/5 mL) and urea[1] (0.601 g/100 mL) were prepared in phosphate buffer (3 mM at pH 6.00). The water soluble test chemical solutions were also prepared in this buffer and serial dilutions made with buffer. The organic chemical stock solutions were prepared in buffer containing 10% acetone. Serial dilutions were then made with the phosphate buffer. The aqueous acetone solvent had no measurable effect on urease activity. In a few cases where relatively high concentrations of chemicals were not in true solution, suspensions were analyzed. The reaction mixture consisted of 2.00 mL of buffer (reference) or 2.00 mL of the test reagent in buffered solution, and 100 µL of the enzyme solution. The preincubation time to allow full interaction between the test chemical and the enzyme was 10 min. The initial pH was recorded (near 6.00) and 1.900 mL of substrate solution was then added with mild stirring. Reaction was allowed to proceed at 22°C for 2.50 min and the final pH then recorded. The control samples showed a pH rise to about 8.50 during this time span. The chemicals which affected the activity of urease caused a pH rise that varied between these limits. The percent of inhibition was then calculated on the basis of the pH value derived from the standard curve and with reference to the lower (eg. 6.00) and upper (eg. 8.50) pH limits. No chemical studied caused a measurable activation of the enzyme. The test chemicals studied are shown in Table 1. All chemicals were reagent grade of established purity.

The components of the inorganic chemical mixture (in buffer) were: $HgCl_2$, $CuCl_2 \cdot 2H_2O$, $HAuCl_4 \cdot 3H_2O$, $CdCl_2 \cdot 2H_2O$, $NiCl_2 \cdot 6H_2O$, Na_2PdCl_4, $CoCl_2 \cdot 6H_2O$, K_2PtCl_6, K_2PdCl_6, and $ZnCl_2$. The mixture of organic chemicals (in buffered 10% aqueous acetone) contained: 2,4-D[2], 2,2-dichloropropionic acid, p-chlorobenzoic acid, Warfarin, acetazolamide, 2,4,5-T, Rootone, allethrin, p-nitrophenol and eserine. The concentration of each component in each stock preparation was based upon that concentration which alone constituted a threshold ("effect-no effect") value. Analyses of these preparations and dilutions of them were then carried out as with single toxicant solutions.

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[1] Jack bean urease (type IX) and urea, Sigma Chemical Company, St. Louis, MO 63178.

[2] See Table 1 for identity of abbreviated chemicals.

TABLE 1

Effect of Chemicals on Urease Activity (I_t = Molar concentration at the threshold of "effect-no effect"; I_{50} = molar concentration causing 50% inhibition. A = antibiotic, F = fungicide, H = herbicide, I = insecticide, P = industrial pollutant, R = rodenticide.

Chemical	I_t	I_{50}	Chemical	I_t	I_{50}
A. Inorganic salts (active cation)			**B. Inorganic salts (active anion)**		
$AgNO_3$	4×10^{-7}	7×10^{-7}	H_2O_2	8×10^{-4}	3×10^{-3}
$AlCl_3$	1×10^{-3}	1×10^{-2}	$NaAsO_2$	1×10^{-4}	2×10^{-3}
$BaCl_2 \cdot 2H_2O$	a		$NaBH_4$	1×10^{-4}	2×10^{-3}
$BeCl_2$	a		NaBr	a	
$BiCl_3$	a		$Na_2Cr_2O_7$	1×10^{-5}	3×10^{-5}
$CaCl_2$	2×10^{-3}	1×10^{-2}	NaCN	3×10^{-3}	4×10^{-2}
$CdCl_2 \cdot 2H_2O$	7×10^{-6}	3×10^{-4}	NaF	8×10^{-5}	5×10^{-4}
$CoCl_2 \cdot 6H_2O$	7×10^{-4}	2×10^{-3}	Na_2HAsO_4	a	
$CrCl_3$	2×10^{-3}	6×10^{-3}	NaI	a	
$CuCl_2 \cdot 2H_2O$	4×10^{-7}	1×10^{-6}	$NaIO_4$	3×10^{-6}	6×10^{-6}
$FeCl_2$	3×10^{-3}	4×10^{-2}	NaN_3	a	
$FeCl_3 \cdot 6H_2O$	a		Na_2SeO_3	2×10^{-4}	1×10^{-3}
$HAuCl_4 \cdot 3H_2O$	4×10^{-6}	1×10^{-5}	Na_2SeO_4	6×10^{-3}	b
$H_2PtCl_6 \cdot 6H_2O$	8×10^{-5}	3×10^{-4}	$Na_2S \cdot 9H_2O$	a	
$HgCl_2$	3×10^{-7}	6×10^{-7}	$Na_2TeO_4 \cdot 2H_2O$	7×10^{-4}	3×10^{-3}
K_2PdCl_6	1×10^{-4}	8×10^{-4}			
LiCl	a				
$MgCl_2$	a				
$MnCl_2$	1×10^{-3}	3×10^{-3}			
$NiCl_2 \cdot 6H_2O$	2×10^{-5}	1×10^{-2}			
NaCl	a				
Na_2PdCl_4	6×10^{-5}	4×10^{-4}			
NH_4Cl	1×10^{-2}	b			
$Pb(NO_3)_2$	6×10^{-5}	2×10^{-4}			
$SnCl_2 \cdot 2H_2O$	a				
$SnCl_4 \cdot 5H_2O$	4×10^{-3}	1×10^{-2}			
$ZnCl_2$	8×10^{-4}	3×10^{-4}			

Chemical	I_t	I_{50}
C. Organometals		
Cacodylic acid (H) : Dimethyl arsinic acid	8×10^{-4}	5×10^{-3}
Me_2SnCl_2 : Dimethyl stannic chloride	4×10^{-4}	b
MeHgCl : Methyl mercuric chloride	2×10^{-7}	1×10^{-6}
PhHgCl : Phenyl mercuric chloride	3×10^{-7}	2×10^{-6}

Chemical	I_t	I_{50}
D. Thiocarbamates		
Eptam (F) : S-Ethyl dipropylthiocarbamate	a	
Thiram (F) : Tetramethylthiuram disulfide	a	
E. Carbamates		
Carbaryl (I) : Sevin; 1-Naphthyl-N-methyl carbamate	a	
Eserine : Physostigmine sulfate	2×10^{-3}	4×10^{-2}
IPC (H) : Isopropyl-N-phenyl carbamate	a	
F. Organophosphates		
Diazinon (I) : O,O-Diethyl-O-(2-isopropyl-4-methyl-6-pyrimidyl)-phosphorothioate	a	
Malathion (I) : S-(1,2-Dicarboxyethyl)-O,O-dimethyldithiophosphate	a	
Maloxon : Diethyl[(dimethoxyphosphinyl)thio]butanedioate	a	
G. Urea derivatives		
Diuron (H) : 3-[3,4-Dichlorophenyl]-1,1-dimethyl urea	a	
Fenuron (H) : 1,1-Dimethyl-3-phenyl urea	a	
Monuron (H) : 3-p-Chlorophenyl-1,1-dimethyl urea	a	
H. Aromatic halohydrocarbons		
Arochlor 1016 (P) : Polychlorobiphenyl	a	a
DDT (I) : Dichlorodiphenyltrichloroethane (o,p and p,p')		
Dicofol (I) : Kelthane;4,4'-Dichloro-a-[trichloromethyl] benzhydrol	a	
I. Aliphatic halohydrocarbons		
Aldrin (I) : 1,2,3,4,10,10-Hexachloro-1,4,4a,5,8,8a-hexahycro-endo-exo-1,4: 5,8-dimethanonaphthalene	a	
Captan (F) : N-Trichloromethylthio)-4-cyclohexane-1,2-dicarboximide	a	
Dieldrin (I) : 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-endo-exo-1,4,:5,8-dimethanonaphthalene	a	
Lindane (I) : 1,2,3,4,5,6-hexachlorocyclohexane (γ-isomer)	a	
J. Alcohols		
Ethanol :	a	
Methanol :	a	

Chemical	I_+	I_{50}
H. Phenols, halophenols and quinones		
p-Benzoquinone	a	
Catechol : 3,4-Dihydroxy benzene	a	
p-Hydroxyquinone	a	
1-Napthol	a	
p-Nitrophenol (P)	8×10^{-3}	b
Pentachlorophenol (F)	1×10^{-3}	2×10^{-3}
Phenol (A)	a	
I. Haloacids and halophenoxyacids		
p-Chlorobenzoic acid	7×10^{-4}	2×10^{-3}
2,4,-D (H) : 2,4-Dichlorophenoxy acetic acid	2×10^{-4}	2×10^{-3}
2,2-Dichloropropionic acid (P)	5×10^{-4}	1×10^{-2}
Iodoacetic acid	7×10^{-4}	2×10^{-3}
2,4,5-T (H) : 2,4,5-Trichlorophenoxy acetic acid	3×10^{-3}	5×10^{-3}
Trichloroacetic acid (P)	a	
M. Miscellaneous chemicals		
Acetazolamide : Diamox;2-Acetylamino-1,3,4-thiadiazole-5-sulfonamide	9×10^{-4}	1×10^{-2}
Acetone	a	
Allethrin (I) : 2,2-Dimethyl-3-(2-methyl propenyl) cyclopropane carboxylic acid ester of 2-Allyl-4-hydroxy-3-methyl-2'-cyclopenten-1-one	5×10^{-3}	b
Aniline	a	
Benzene	a	
Caffeine : 1,3,7-Trimethyl xanthine	a	
EDTA : Ethylene diamine tetraacetic acid ($2H_2O \cdot 2Na$)	6×10^{-3}	b
MS-222 : Ethyl-*m*-aminobenzoate methanesulfonic acid	a	
NaDDS : Sodium dodecyl sulfate	4×10^{-4}	8×10^{-4}
NAD : Nicotinamide adenine dinucleotide (oxidized)	a	
NADH : Nicotinamide adenine dinucleotide (reduced)	3×10^{-3}	2×10^{-2}
Piperonyl butoxide : 3,4-Methylenedioxy-6-propylbenzyl-n-butyl diethylene glycol ether	a	
Rootone (H) : 1-Napthaleneacetamide	5×10^{-3}	b
Warfarin (R) : 3-Acetonylbenzyl-4-hydroxycoumarin		

[a] Negligible effect at 10^{-2}M.

[b] $I_{50} > 5 \times 10^{-2}$M.

RESULTS AND DISCUSSION

The results of the analysis of the 93 various chemicals for effect on urease activity are given in Table 1. These data are expressed in terms of I_t (molar concentration at the threshold effect level) and I_{50} (molar concentration causing 50% inhibition). These inhibition indices were derived from graphing each test reagent in terms of percent inhibition vs. concentration of test reagent in the reaction mixture. Each analysis was carried out in triplicate and mean values used to prepare the plot. Concentrations of the test reagents were adjusted so as to cover the range from 0 to 100% inhibition or to that degree of inhibition caused by the highest concentration studied, which was 5×10^{-2}M.

On the basis of I_t values, the results indicated that the organometallic chemicals, methyl mercuric chloride and phenyl mercuric chloride, were the strongest inhibitors studied, which was about 10^7 times more inhibiting than NaCl. Also highly inhibiting were Hg(II), Ag(I) and Cu(II), followed by IO_4^{-1}, Au(III), and Cd(II). Of the anions studied, in addition to IO_4^{-1} and $Cr_2O_7^{-2}$, F^{-1}, AsO_2^{-1}, and BH_4^{-1} had a moderate effect which was more inhibiting than SeO_3^{-1} and several other ions. Some anions, Br^{-1}, I^{-1}, N_3^{-1}, and S^{-2}, showed no measureable effect on urease activity.

Of the organic chemicals studied, the herbicide, 2,4-D, was found to be most reactive, followed closely by sodium dodecylsulfate, 2,2,-dichloropropionic acid and p-chlorobenzoic acid. It is possible that these organic chemicals may affect biota metabolism through inhibition of this enzyme if it is present in the tissue. Although urease is not a common component of higher animals, it is present in a variety of plants, bacteria, algae and microorganisms (Reithel 1971). The herbicides derived from urea (diuron, monuron and fenuron) were found to be unreactive. Selenite was more inhibiting than selenate; Pd(II) more than Pd(IV); arsenite more than arsenate; Cr(VI) more than Cr(III); and Fe(II) more than Fe(III). None of the widely used pesticides such as malathion, captan, diazinon and carbaryl elicited a measurable effect. Warfarin (rodenticide) and pentachlorophenol (fungicide) caused a mild effect.

Some foreign chemicals possessing similar chemical and physical properties may "add" with regard to effect upon the activity of those enzymes which do not show absolute substrate specificity. It is known, for example, that certain combinations of drugs and other chemicals involved in human medicine "add" in pharmacological effect, whereas, other combinations "subtract" in effect. This was found concerning the two preparations of mixtures, whereby both preparations of 10 components, each caused about a 10-fold increase in inhibition.

Much research remains to be done in order to understand fully the biological consequences to all living components of an ecosystem when there is exposure to foreign chemicals in the environment, acting singly or in combination, over a short or a long-term, and with or without influence from other stressors.

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